ICMSF methods studies. II. Comparison of analytical schemes for detection of *Salmonella* in high-moisture foods[1,2,3]

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Twenty-one naturally contaminated high-moisture food samples were analyzed to compare the relative efficiency of using thirteen 25-g, three 108.3-g, one 325-g, and one 500-g subsamples in recovery of salmonellae. In addition, recovery by preenrichment in lactose broth was compared with direct selective enrichment in selenite cystine and tetrathionate brilliant green broths. Analysis of preenriched large lumped subsamples, prepared by combining multiple 25-g subsamples, detected positive samples as effectively as the analysis of individual 25-g subsamples. In the analysis of 25-g subsamples, direct enrichment detected as many positive "lots" as preenrichment, but in the analysis of lumped subsamples, direct enrichment gave dramatically reduced recovery. Nevertheless, directly enriched 25-g subsamples resulted in a significantly lower rate of recovery than preenriched subsamples. The results indicate that analysis of a single lumped 325-g preenriched subsample is as efficient in the recovery of salmonellae as the analysis of thirteen 25-g preenriched subsamples. Further, it is apparent that preenrichment of high-moisture foods, such as meat, poultry, and eggs is to be recommended over direct enrichment for the detection of salmonellae.

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On a analysé vingt et un échantillons d'aliments à forte teneur d'humidité naturellement contaminés dans le but de comparer, lors de la recherche de salmonelles, l'efficacité relative entre treize prises d'essai de 25-g, trois de 108.3-g, une de 325-g et une de 500-g. On a aussi comparé l'isolement par pré-enrichissement en bouillon lactosé par rapport à l'enrichissement direct sélectif en bouillon sélénite-cystine ou tétrathionate-vert brillant. En analysant un échantillon pré-enrichi en bloc en combinant plusieurs prises d'essai de 25-g, on peut détecter des échantillons positifs avec autant de satisfaction qu'en analysant individuellement des prises d'essai de 25-g. A l'analyse des prises de 25-g, l'enrichissement direct permet de détecter autant de "lots" positifs que par pré-enrichissement; lorsque les prises sont analysées en bloc l'enrichissement direct ne permet qu'une très faible récupération. Néanmoins, il y a un taux de récupération significativement plus bas dans les prises d'essai de 25-g directement enrichies par rapport aux prises préenrichies. Les résultats indiquent qu'une prise d'essai unique préenrichie en bloc de 325-g est aussi efficace pour mettre les salmonelles en évidence que l'analyse de treize prises de 25-g préenrichies. Il ressort de plus que dans le cas d'aliments à forte teneur d'humidité soit la viande, la volaille, les oeufs, le préenrichissement est à recommander par rapport à l'enrichissement direct dans la détection des salmonelles. [Traduit par le journal]

Silliker and Gabis (7) reported that *Salmonella*-positive lots of low-moisture foods could be detected with equal certainty by analysis of sixty 25-g individual, fifteen 100-g, or three 500-g lumped samples. The purpose of the present investigation was to determine whether multiple 25-g subsamples could be similarly lumped to provide large sample units for the analysis of high-moisture foods, such as meats, poultry, and eggs. The economic advantage of lumping samples is obvious; indeed, this approach makes statistical quality control over the *Salmonella* defect economically feasible, particularly for high-risk foods where analysis of as many as sixty 25-g subsamples has been recommended (5). The United States Food and Drug Administration (12) has officially recognized the lumping of multiple 25-g samples to produce 375-g samples for analysis.

It is generally recognized that the detection of salmonellae in high-moisture foods presents problems different from those encountered in the analysis of dried products. In particular, meats, poultry, and eggs are apt to contain large num-

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bers of competing microorganisms. Consequently, a number of the recommended procedures for the analysis of such foods specify direct selective enrichment in contrast to preenrichment which is used in the analysis of dried foods (1, 3, 10, 12). Recently, however, Edel and Kampelmacher (4) and Moran (personal communication) reported that preenrichment of meats in a nonselective medium results in greater sensitivity of *Salmonella* detection than does direct selective enrichment. In the present work, the efficacy of lumping subsamples was investigated in conjunction with the influence of preenrichment as compared to direct selective enrichment.

Materials and Methods

Source of Samples

All samples were frozen raw products. Skeletal meat samples were obtained from human food processors. Organ meat and organ–skeletal meat mixtures were obtained from a pet food processor. Salmonellae in these samples were natural contaminants.

Sample Preparation

Meat, poultry, and casing samples were passed once through a meat grinder with a plate containing holes 8 mm in diameter. The samples were then hand mixed and reground twice through the meat grinder with a plate containing holes 3 mm in diameter. Egg samples were mixed thoroughly by hand in a 20-liter can using a large ladle. Mixing of the samples was a preliminary step to analysis to promote even distribution of contaminating organisms within the samples as received.

Twenty-five-gram subsamples were then weighed from each mixed sample in sufficient number for analysis. The 25-g subsamples were randomly selected to form lumped samples as described below.

Sampling

The acceptance criteria for category III products in the NRC report (5) was followed. Products in this category are accepted if thirteen 25-g subsamples are analyzed for *Salmonella* and found negative. Accordingly, 13 of the 25-g units were used for analysis as individual subsamples. Three lumped subsamples of 108.3 g each were prepared by combining four 25-g units and weighing an additional 8.3-g subsample from one of the 25-g units. A 325-g lumped subsample was prepared by combining thirteen 25-g units. In addition, a 500-g subsample was included in view of our previous experience with the analysis of 500-g dry food samples (7). Thus for each analytical procedure, thirteen 25-g, three 108.3-g, one 325-g, and one 500-g subsamples were analyzed.

Enrichment Procedures

All samples for non-selective preenrichment were introduced into sufficient volumes of lactose broth containing 1% (v/v) Tergitol Anionic 7 (sodium heptadecyl sulfate, Union Carbide, Chicago) to obtain a 1:10 dilution of the sample (7). For direct selective enrichment, the samples were introduced into appropriate volumes of tetrathionate broth containing brilliant green dye and selenite cystine broth (7, 10). All media were obtained from Difco, Detroit, Michigan. Preenrichment and direct enrichment cultures were incubated 24 h at 35C.

Subculturing of Enrichment Cultures

One-milliliter portions of the lactose preenrichment cultures were transferred to 9 ml of tetrathionate broth and 9 ml of selenite cystine broth, and these cultures were incubated 24 h at 35C. After incubation, the selective enrichment cultures, originating both from lactose preenrichment cultures and direct enrichment, were streaked onto brilliant green (BG), *Salmonella-Shigella* (SS), and bismuth sulfite (BS) agar plates which were then incubated at 35C. The BS and SS plates were examined after 24 h and the BS plates after 48 h.

Identification of Salmonella

Suspect colonies from the selective-differential agar plates were identified by conventional biochemical and serological procedures (10). Serological identification was accomplished using slide agglutinations with group-specific O antisera and tube agglutinations using Spicer-Edwards H antisera (Difco).

Results

The *Salmonella*-recovery patterns of 21 high-moisture food samples are summarized in Table 1. All samples contained *Salmonella*; however, none of the samples was positive for salmonellae by all methods. Lactose preenrichment of three 108.3-g subsamples of each sample resulted in the detection of salmonellae in 18 of the 21 samples. Eighteen positive samples were also detected using direct enrichment in selenite broth of thirteen 25-g subsamples. Preenrichment of thirteen 25-g subsamples, as well as a single 500-g lumped subsample effected the detection of 17 positive samples. Lactose preenrichment of a single 325-g subsample from each sample resulted in the detection of only 14 positive samples.

Direct enrichment in tetrathionate broth was far less sensitive than direct enrichment in selenite broth. The use of 25-g subsamples inoculated directly into tetrathionate broth detected 13 positive samples, but with increasing weights of lumped samples the sensitivity decreased dramatically. With both 325 and 500-g lumped subsamples direct enrichment in tetrathionate broth resulted in the detection of only 2 of 21 positive samples. A similar decrease in sensitivity was noted with lumped samples inoculated directly into selenite broth.

Table 2 summarizes detailed recovery data for each of the enrichment procedures, namely

TABLE 1

Patterns in the recovery of salmonellae by three different sampling and analytical methods

Products	Lactose preenrichment*				Direct selective enrichment							
					Tetrathionate				Selenite			
	Wt. of aliquots, g				Wt. of aliquots, g				Wt. of aliquots, g			
	25	108.3	325	500	25	108.3	325	500	25	108.3	325	500
	No. of aliquots				No. of aliquots				No. of aliquots			
	13	3	1	1	13	3	1	1	13	3	1	1
Beef trimmings	−	−	−	−	−	−	−	−	+	−	−	−
Beef liver	+	+	+	+	+	+	+	−	+	+	+	+
Beef liver	+	+	+	+	+	+	−	+	+	−	−	−
Beef kidney	+	+	+	+	+	+	−	−	+	+	−	−
Pork trimmings	+	+	+	+	+	−	−	−	+	+	−	−
Pork lungs	−	−	−	+	−	−	−	−	+	+	+	+
Pork liver	+	+	+	+	+	+	−	+	+	+	−	−
Pork liver	+	+	+	+	+	−	−	−	+	+	−	−
Pork kidney	+	+	+	+	+	+	+	−	+	+	−	−
Pork kidney	+	+	+	+	+	+	−	−	+	+	+	+
Pork kidney	+	+	+	+	+	−	−	−	+	+	−	−
Pork spleen	−	+	−	−	−	−	−	−	+	−	−	−
Pork spleen	+	+	+	+	+	−	−	−	+	+	+	−
Hog casings (unsalted)	+	+	+	+	−	−	−	−	+	−	−	+
Chicken (whole)	+	+	+	+	+	−	−	−	+	+	+	+
Chicken (whole)	+	+	−	+	−	−	−	−	+	−	−	−
Chicken (whole)-liver (beef)	+	+	+	+	+	−	−	−	+	+	+	+
Chicken (whole)-liver (beef)	+	+	+	+	+	−	−	−	+	+	−	−
Whole egg	+	−	−	−	−	−	−	−	−	−	−	−
Whole egg	−	+	−	−	−	−	−	−	−	−	−	−
Egg albumen	+	+	−	+	−	−	−	−	−	−	−	−
Total samples positive	17	18	14	17	13	6	2	2	18	13	6	6

*Followed by selective enrichment.

TABLE 2

Influence of sample size and enrichment procedure in the recovery of salmonellae from high-moisture foods

Products	Lactose preenrichment*				Direct selective enrichment: Tetrathionate				Direct selective enrichment: Selenite				Minimum estimated level/125 g
	Wt. of aliquots, g				Wt. of aliquots, g				Wt. of aliquots, g				
	25	108.3	325	500	25	108.3	325	500	25	108.3	325	500	
	No. of aliquots				No. of aliquots				No. of aliquots				
	13	3	1	1	13	3	1	1	13	3	1	1	
Beef trimmings	0	0	0	0	0	0	0	0	1	0	0	0	<0.08
Beef liver	13	3	1	1	4	3	1	0	7	2	1	1	NA
Beef liver	13	1	1	1	3	3	0	1	2	0	0	0	NA
Beef kidney	5	2	1	1	3	1	0	0	4	1	0	0	NA
Pork trimmings	9	3	1	1	3	0	0	0	7	2	0	0	NA
Pork lungs	0	0	0	1	0	0	0	0	2	1	1	1	0.08
Pork liver	12	2	1	1	7	1	0	1	11	1	0	0	NA
Pork liver	3	3	1	1	4	0	0	0	11	1	0	0	NA
Pork kidney	13	3	1	1	12	2	1	0	1	3	0	0	NA
Pork kidney	13	3	1	1	12	3	0	0	10	3	1	1	NA
Pork kidney	12	3	1	1	1	0	0	0	5	2	0	0	NA
Pork spleen	0	1	0	0	0	0	0	0	1	0	0	0	0.08
Pork spleen	13	3	1	1	3	0	0	0	7	2	1	0	NA
Hog casings (unsalted)	1	2	1	1	0	0	0	0	2	0	0	1	NA
Chicken (whole)	8	3	1	1	4	0	0	0	7	1	1	1	NA
Chicken (whole)	2	1	0	1	0	0	0	0	1	0	0	0	0.34
Chicken (whole)-liver (beef)	12	3	1	1	7	0	0	0	8	3	1	1	NA
Chicken (whole)-liver (beef)	6	2	1	1	2	0	0	0	6	2	0	0	NA
Whole egg	1	0	0	0	0	0	0	0	0	0	0	0	0.08
Whole egg	0	1	0	0	0	0	0	0	0	0	0	0	0.08
Egg albumen	1	1	0	1	0	0	0	0	0	0	0	0	0.08
Total samples positive	137	40	14	17	65	13	2	2	93	24	6	6	
Total samples analyzed	273	63	21	21	273	63	21	21	273	63	21	21	
% positive	50.2	63.5	66.7	81.0	23.8	20.6	9.5	9.5	34.1	38.1	28.6	28.6	

*Followed by selective enrichment.

NOTE: NA = not applicable.

lactose preenrichment, direct enrichment in tetrathionate broth, or direct enrichment in selenite broth. An estimation of the level of salmonellae has been made for samples with a low level of contamination. A total of 1475 g of each product was analyzed using lactose preenrichment. Assuming that each positive result from the lightly contaminated subsample was derived from a single *Salmonella*, then from the total number of positive subsamples from that product an estimated minimum *Salmonella* level per 125 grams may be calculated. For example, in the second chicken sample there were two positives in the 25-g subsamples, none in the 325-g, and one in the 500-g subsample. The estimated minimum number of salmonellae was calculated to be 0.34 per 125 grams based on four positives in 1475 g of product (4:1475:X:125). The estimation is made for the level of *Salmonella* in 125 g, since according to the acceptance criteria in the NRC report (5), negative results of thirteen 25-g subsamples indicates a probability of about 95% that the level of contamination is no greater than one organism in 125 g. Stated differently, only one time in 20 should there be failure to isolate *Salmonella* from a sample containing one organism in 125 g, if 325 g of the sample is analyzed.

When 25-g subsamples were used, no sample containing >1 organism in 125 g was found negative by any of the three enrichment procedures. Further, when lactose preenrichment was used no sample containing >1 *Salmonella* in 125 g was found negative by the other three sampling procedures, i.e., three lumped 108.3-g or single lumped 325-g and 500-g subsamples. The results for lumped samples directly enriched in tetrathionate and selenite broths, however, were quite different. Nine samples containing >1 *Salmonella* in 125 g were negative in one or more instances in which lumped samples were directly enriched in tetrathionate broth. Similarly, six of the samples containing >1 *Salmonella* in 125 g were found negative in one or more cases when lumped samples were enriched directly in selenite broth.

From the foregoing it may be concluded that if the level of salmonellae in a given sample is >1 cell in 125 g, one may expect to detect that sample as positive if thirteen 25-g subsamples are analyzed by either lactose preenrichment or direct enrichment in either tetrathionate or selenite broth. It would be expected that the sample would also be found positive if a like amount of product were analyzed using three lumped 108.3-g subsamples or one lumped 325-g subsample, but only if these lumped subsamples were preenriched. The data indicate that analysis of such lumped subsamples through direct enrichment in either tetrathionate or selenite broths would give a significant number of negative results.

This perplexing loss of sensitivity when lumped samples were directly enriched was studied further. Consideration was given to the fact that 13 sets of differential plates were streaked when 25-g subsamples were enriched; whereas only three sets were inoculated from the three 108.3-g lumped subsamples and only one set from the 325-g lumped subsample. Accordingly, three samples (pork liver, pork spleen, and chicken), each of which was naturally contaminated with salmonellae, were inoculated directly into tetrathionate broth. Instead of subculturing the usual three sets of plates from the three 108.3-g subsamples, four sets of plates were streaked from two of the tetrathionate enrichment cultures and five from the third, giving a total of 13 sets of differential plates from the three 108.3-g subsamples. Similarly, 13 sets of plates were streaked from the single 325-g lumped subsample. Thus, the same number of differential agar plates were examined for each series of subsamples. Recovery results gave no indication that the inferior recovery of salmonellae from lumped 108.3-g and 325-g subsamples could be overcome by streaking 13 instead of 3 sets of plates.

The possibility that the use of lumped subsamples resulted in overgrowth of non-salmonellae was investigated. Quantitative determinations of total Enterobacteriaceae were carried out (10) on each of the tetrathionate cultures. There was no significant difference between the numbers of Enterobacteriaceae in the incubated 25-g subsamples and those for either the 108.3 and 325-g subsamples. Furthermore, there was no indication of significantly different levels of Enterobacteriaceae in the *Salmonella*-positive tetrathionate cultures compared with those in the *Salmonella*-negative cultures. Similar experiments were not conducted on samples directly enriched in selenite broth. The loss in sensitivity resulting from the direct inoculation of selenite and tetrathionate broths remains unexplained.

Whereas *Salmonella*-positive samples containing >1 cell in 125 g can be detected as posi-

tive if thirteen 25-g subsamples are analyzed either by lactose preenrichment or by direct enrichment in either tetrathionate or selenite broths, a striking aspect of the results is the greater sensitivity of the lactose preenrichment procedure. In considering the 25-g subsamples only, 50.2% of the 273 meat samples were found positive using preenrichment. In contrast, direct enrichment in tetrathionate and selenite broths resulted in only 23.8% and 34.1% positive tests, respectively. The difference between preenrichment followed by selective enrichment and selective enrichment without preenrichment is highly significant ($P < 0.001$).

Discussion

The results clearly indicate that lumping of 25-g subsamples to produce larger samples for analysis is as sensitive for the detection of positive samples as is the analysis of individual 25-g subsamples. This finding parallels the results of our previous study (7) on dried foods. The importance of this is obvious and has been discussed.

The present findings indicate that high-moisture foods should be preenriched in a non-selective medium. This is generally not done in the analysis of such products as eggs, meat, and poultry (1, 2, 10). The first edition of *Recommended methods for the microbiological examination of foods* (1), published in 1958 by the American Public Health Association (APHA), prescribes direct enrichment of food in selenite broth. This, however, was before North's (6) recognition that preenrichment of dried egg albumen in lactose broth increased subsequent sensitivity of direct enrichment procedures. Since that time, the advantage of preenrichment of dried foods has been widely appreciated. The second edition of the APHA book on microbiological methods (2), published in 1966, suggests a possible advantage of preenriching dried eggs and other dehydrated foods but makes no mention of enrichment procedures for high-moisture foods. Thatcher and Clark (10) indicate that non-selective enrichment is not required for raw meat, poultry, and unpasteurized eggs. The *Microbiology laboratory guidebook* of the United States Department of Agriculture (11), published in 1969, states that ordinarily raw meat is inoculated directly into tetrathionate broth, but adds parenthetically "a lactose pre-enrichment step may be used at the discretion of the investigator." A private communication (A. Moran, USDA) indicates that the USDA laboratories have, in fact, practiced non-selective enrichment in lactose broth since 1967. Published reports from USDA laboratories indicate that non-selective enrichment is used (8, 9). The latest edition of the *Bacteriological analytical manual* of the United States Food and Drug Administration (3) states that raw foods and finished products that have been grossly contaminated after processing should be examined by direct enrichment in selective broths.

Edel and Kampelmacher (4) reported that in the analysis of 150 naturally contaminated meat samples, 91.6% of the total number of positive samples were detected as positive by preenrichment in buffered peptone water, followed by selective enrichment in tetrathionate broth containing brilliant green, the latter incubated at 43C. On the other hand, only 69.4% of the total positive samples were detected by direct enrichment in tetrathionate broth incubated at 43C. The present results are in agreement with those of Edel and Kampelmacher. Clearly the examination of raw meats, poultry, and eggs for salmonellae should involve preenrichment of samples in a non-selective medium.

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